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<p>(54) Title: CHEMOKINES THAT INHIBIT IMMUNODEFICIENCY VIRUS INFECTION AND METHODS BASED THEREON (57) Abstract The present invention relates to therapeutic compositions and methods for treating and preventing infection by an immunodeficiency virus, particularly HIV infection, using chemokine proteins, nucleic acids and/or derivatives or analogues thereof.</p>		

**CHEMOKINES THAT INHIBIT IMMUNODEFICIENCY  
VIRUS INFECTION AND METHODS BASED THEREON**

**1. INTRODUCTION**

The present invention relates to therapeutic  
5 compositions and methods for treating and preventing  
infection by an immunodeficiency virus, particularly HIV  
infection, using chemokine proteins, nucleic acids and/or  
derivatives or analogues thereof.

**2. BACKGROUND OF THE INVENTION**

Human immunodeficiency virus (HIV) induces a persistent  
and progressive infection leading, in the vast majority of  
cases, to the development of the acquired immunodeficiency  
syndrome (AIDS) (Barre-Sinoussi et al., 1983, Science 220:  
15 868-870; Gallo et al., 1984, Science 224:500-503). There are  
at least two distinct types of HIV: HIV-1 (Barre-Sinoussi et  
al., 1983, Science 220:868-870; Gallo et al., 1984, Science  
224:500-503) and HIV-2 (Clavel et al., 1986, Science 233:343-  
346; Guyader et al., 1987, Nature 326:662-669). In humans,  
20 HIV replication occurs prominently in CD4<sup>+</sup> T lymphocyte  
populations, and HIV infection leads to depletion of this  
cell type and eventually to immune incompetence,  
opportunistic infections, neurological dysfunctions,  
neoplastic growth, and ultimately death.

25 HIV is a member of the lentivirus family of retroviruses  
(Teich et al., 1984, RNA Tumor Viruses, Weiss et al., eds.,  
CSH-press, pp. 949-956). Retroviruses are small enveloped  
viruses that contain a single-stranded RNA genome, and  
replicate via a DNA intermediate produced by a virally-  
30 encoded reverse transcriptase, an RNA-dependent DNA  
polymerase (Varmus, H., 1988, Science 240:1427-1439). Other  
retroviruses include, for example, oncogenic viruses such as  
human T-cell leukemia viruses (HTLV-1, -II, -III), and feline  
leukemia virus.

35 The HIV viral particle consists of a viral core,  
composed in part of capsid proteins designated p24 and p18,  
together with the viral RNA genome and those enzymes required

late stages of aids (Weiss et al., 1996, Science 272:1885-1886). The majority of primary HIV-1 isolates (i.e., viruses not extensively passaged in culture) replicate efficiently in primary lymphocytes, monocytes and macrophages, but grow 5 poorly in established T cell lines. These isolates have been termed M-tropic. The viral determinant of T- and M- tropism maps to alterations in the third variable region of gp120 (the V3 loop) (Choe et al., 1996, Cell 85:1135-1148; Cheng-Mayer et al., 1991, J. Virol. 65:6931-6941; Hwang et al., 10 1991, Science 253:71-74; Kim et al., 1995, J. Virol., 69:1755-1761; and O'Brien et al., 1990, Nature 348:69-73). The characterization of HIV isolates with distinct tropisms taken together with the observation that binding to the CD4 cell surface protein alone is insufficient to lead to 15 infection, suggest that cell-type specific cofactors might be required in addition to CD4 for HIV-1 entry into the host cell.

Recently, certain chemokines produced by CD8<sup>+</sup> T cells have been implicated in suppression of HIV infection. The 20 chemokines RANTES (regulated on activation normal T cell expressed and secreted), macrophage-inflammatory protein-1 $\alpha$  and -1 $\beta$  (MIP-1 $\alpha$  and MIP-1 $\beta$ , respectively), which are secreted by CD8<sup>+</sup> T cells, were shown to suppress HIV-1 p24 antigen production in cells infected with HIV-1 or HIV-2 isolates in 25 vitro (Cocchi et al., 1995, Science 270:1811-1815).

Additionally, high levels of these chemokines have been found to be secreted by CD4<sup>+</sup> T lymphocytes in individuals that have been exposed to HIV-1 on multiple occasions but, remain uninfected (Paxton et al., 1996, Nature Med. 2:412-417). 30 While RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$  alone or in combination, potently suppress a variety of primary HIV-1 isolates and macrophage tropic isolates, such as HIV-1<sub>MAC</sub>, some established laboratory strains, such as HIV-1<sub>IIIB</sub>, are refractory to inhibition of infection or replication by these chemokines 35 (Cocchi et al., 1995, Science 270:1811-1815).

Chemokines, or chemoattractant cytokines, are a subgroup of immune factors that have been shown to mediate chemotactic

(See, reviews by Horuk, R., 1994, Trends Pharmacol. Sci. 15:159-165; and Murphy, P.M., 1994, Annu. Rev. Immunol. 12:593-633). Competition binding and cross-desensitization studies have shown that chemokine receptors exhibit

5 considerable promiscuity in ligand binding. Examples demonstrating the promiscuity among  $\beta$  chemokine receptors include: CC CKR-1, which binds RANTES and MIP-1 $\alpha$  (Neote et al., 1993, Cell 72: 415-425), CC CKR-4, which binds RANTES, MIP-1 $\alpha$ , and MCP-1 (Power et al., 1995, J. Biol. Chem.

10 270:19495-19500), and CC CKR-5, which binds RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$  (Alkhatib et al., 1996, Science, in press and Dragic et al., 1996, Nature 381:667-674). Erythrocytes possess a receptor (known as the Duffy antigen) which binds both  $\alpha$  and  $\beta$  chemokines (Horuk et al., 1994, J. Biol. Chem.

15 269:17730-17733; Neote et al., 1994, Blood 84:44-52; and Neote et al., 1993, J. Biol. Chem. 268:12247-12249). Thus the sequence and structural homologies evident among chemokines and their receptors allows some overlap in receptor-ligand interactions.

20 CC CKR-5 is the major coreceptor for macrophage-tropic strains of HIV-1 (Alkhatib et al., 1996, Science, in press; Choe et al., 1996, Cell 85:1135-1148; Deng et al., 1996, Nature 381:661-666; Doranz et al., 1996, Cell 85:1149-1158; Dragic et al., 1996, Nature 381:667-674). RANTES, MIP-1 $\alpha$ , or

25 MIP-1 $\beta$ , the chemokine ligands for this receptor have been shown to block HIV Env-mediated cell fusion directed by CC CKR-5 (Alkhatib et al., 1996, Science, in press; and Dragic et al., 1996, Nature 381:667-674). Additional support for the role of CC CKR-5 as an M-tropic HIV-1 cofactor comes from

30 the finding that a 32-base pair deletion in the CC CKR-5 gene found in three multiply exposed but uninfected individuals, prevents HIV from infecting macrophages (Liu et al., 1996, Cell 86:367-377). However, only three of the 25 uninfected individuals studied had this mutation.

35 The V3 loop of gp120 is the major determinant of sensitivity to chemokine inhibition of infection or replication (Cocchi et al., 1996, Nature Medicine 2:1244-

to been active against HIV (Mitsuya et al., 1991, Science 249:1533-1544).

The new treatment regimens for HIV-1 show that a combination of anti-HIV compounds, which target reverse transcriptase (RT), such as azidothymidine (AZT), lamivudine (3TC), dideoxyinosine (ddi), dideoxycytidine (ddc) used in combination with an HIV-1 protease inhibitor have a far greater effect (2 to 3 logs reduction) on viral load compared to AZT alone (about 1 log reduction). For example, impressive results have recently been obtained with a combination of AZT, ddI, 3TC and zidovudine (Perelson et al., 1996, Science 271:1582-1586). However, it is likely that long-term use of combinations of these chemicals will lead to toxicity, especially to the bone marrow. Long-term cytotoxic therapy may also lead to suppression of CD8<sup>+</sup> T cells, which are essential to the control of HIV, via killer cell activity (Blazevic et al., 1995, AIDS Res. Hum. Retroviruses 11:1335-1342) and by the release of factors which inhibit HIV infection or replication, notably the chemokines Rantes, MIP-1 $\alpha$  and MIP-1 $\beta$  (Cocchi et al., 1995, Science 270:1811-1815). Another major concern in long-term chemical anti-retroviral therapy is the development of HIV mutations with partial or complete resistance (Lange, J.M., 1995, AIDS Res. Hum. Retroviruses 10:S77-82). It is thought that such mutations may be an inevitable consequence of anti-viral therapy. The pattern of disappearance of wild-type virus and appearance of mutant virus due to treatment, combined with coincidental decline in CD4<sup>+</sup> T cell numbers strongly suggests that, at least with some compounds, the appearance of viral mutants is a major underlying factor in the failure of AIDS therapy.

Attempts are also being made to develop drugs which can inhibit viral entry into the cell, the earliest stage of HIV infection, by focusing on CD4, the cell surface receptor for HIV. Recombinant soluble CD4, for example, has been shown to inhibit infection of CD4<sup>+</sup> T cells by some HIV-1 strains (Smith et al., 1987, Science 238:1704-1707). Certain primary HIV-1

Citation of a reference hereinabove shall not be construed as an admission that such reference is prior art to the present invention.

5                                   3. SUMMARY OF THE INVENTION

The present invention relates to prophylactic and therapeutic methods and compositions based on chemokine proteins, nucleic acids, derivatives or analogues thereof that inhibit replication and/or infection of an  
10 immunodeficiency virus in vitro or in vivo, decrease viral load, and/or treating or preventing diseases and disorders associated with infection of an immunodeficiency virus. In specific embodiments, the immunodeficiency virus inhibited by the methods and compositions of the invention is HIV.

15       According to the present invention, different chemokine receptors are involved in immunodeficiency virus infection, depending on the particular isolate. The present invention provides methods of identifying the particular chemokine(s) capable of inhibiting the infection or replication of a viral  
20 isolate of a particular patient and of treating such patient. Pharmaceutical compositions comprising chemokines heretofore unknown to be active against HIV are also provided, as well as related methods of treatment or prophylaxis.

The invention also relates to chemokine derivatives or  
25 analogue(s) that bind to a plurality of chemokine receptors and that are effective at preventing diseases or disorders associated with infection of an immunodeficiency virus, particularly HIV infection. The invention also relates to pharmaceutical compositions containing such therapeutically  
30 and prophylactically effective chemokine derivatives or analogues, or the nucleic acids encoding such. In one embodiment, the chemokine derivative or analogue binds to one or more  $\beta$  chemokine receptors selected from a group consisting of CC CKR-1, CC CKR-2A, CC CKR-2B, CC CKR-3, CC  
35 CKR-4 and CC CKR-5. In a preferred embodiment, the derivative or analogue binds to the chemokine receptor CC CKR-5. In another embodiment, the chemokine derivative or

$\alpha$  chemokine, or nucleic acid encoding an  $\alpha$  chemokine, selected from the group consisting of  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, or a therapeutically effective derivative or analogue thereof. In yet another  
5 embodiment, the pharmaceutical composition of the invention contains a combination of  $\alpha$ ,  $\beta$  and/or  $\gamma$  chemokines, nucleic acids encoding  $\alpha$ ,  $\beta$  and/or  $\gamma$  chemokines, or therapeutically or prophylactically effective derivatives or analogues thereof.

10 The present invention also relates to therapeutic compositions based on chemokines and nucleic acids encoding chemokines. Therapeutic compounds of the invention include but are not limited to chemokines, nucleic acids encoding chemokines, and derivatives (including fragments and  
15 chimerics) and analogues thereof, that are effective at inhibiting replication or infection by an immunodeficiency virus.

The invention further relates to therapeutic methods for treatment and prevention of diseases and disorders associated  
20 with infection with an immunodeficiency virus, in particular HIV infection, by administering a therapeutic composition of the invention. More specifically, the invention provides methods for formulating and administering pharmaceutical compositions of the invention that inhibit infection or  
25 replication of one or more known isolates of an immunodeficiency virus, preferably of HIV.

The invention further provides methods for inhibiting the infection or replication of an immunodeficiency virus isolate, in particular, an HIV isolate. In a preferred  
30 embodiment, the invention provides methods for formulating, on a patient-to-patient basis, a therapeutic composition of the invention for treating diseases and disorders associated with the immunodeficiency virus isolate(s) present in an individual at a given time. Methods for administering the  
35 prophylactic or therapeutic compositions of the invention are also provided.

surface protein. This binding induces conformational changes in the envelope protein that increase the exposure of the gp120 V3 loop. The exposed V3 loop then binds to a chemokine receptor, an event that itself triggers further

5 conformational changes leading to fusion and entry of the virus. Depending on their origin (macrophage, CD4<sup>+</sup> PBL, T cell line) various isolates of HIV-1 display a requirement for a distinct array of chemokine receptors. The envelope sequence and structure of a given isolate most likely governs

10 which receptor(s) or receptor array is required for entry into the target cell. It is likely that the envelope molecules share a structural homology with chemokines that allows them to interact with various chemokine binding domains.

15 The Inventors believe that  $\alpha$ -chemokines and  $\beta$ -chemokines other than RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$  (previously demonstrated by the Inventors to inhibit the infection or replication of HIV-1) will also be able to inhibit the infection or

20 replication of HIV-1, depending upon the GCR requirement of the isolate and the target cell. Accordingly, the invention provides methods for formulating pharmaceutical compositions containing one or more chemokines, nucleic acids encoding chemokines, and/or therapeutically and prophylactically effective derivatives or analogues thereof or nucleic acids

25 encoding the same, that will target and treat diseases and disorders associated with infection by an immunodeficiency virus isolate of interest, particularly a primary HIV isolate. Additionally, the invention provides methods for formulating pharmaceutical compositions which contain

30 (preferably as the only chemokines) the chemokines, nucleic acids encoding chemokines, therapeutically and prophylactically effective derivatives or analogues thereof and/or nucleic acids encoding the same, that treat diseases and disorders associated with isolates of immunodeficiency

35 viruses present in an individual at a given time, by a method comprising testing HIV recovered from the individual for inhibition by one or more, preferably a panel of at least 2,

or analogue of one or more  $\alpha$ ,  $\beta$ , or  $\gamma$  chemokines, for treatment and prevention of disorders associated with HIV infection. In a specific embodiment, such composition comprises a therapeutically or prophylactically effective amount of a derivative or analogue of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, or lymphotactin. In another embodiment, such pharmaceutical composition further comprises a therapeutically or prophylactically effective amount of a chemokine, derivative or analogue thereof, selected from the group consisting of RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , IL-8, and/or SDF-1. Pharmaceutical compositions comprising nucleic acids encoding such chemokines, derivatives or analogues are also provided.

In specific embodiments, the pharmaceutical composition comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 chemokines, derivatives or analogues, or nucleic acids encoding the same.

Embodiments in which the pharmaceutical composition of the invention comprises a plurality of chemokines, derivatives and/or analogues selected from those listed above are further described in Section 4.1, *infra*.

The pharmaceutical composition of the invention optionally further comprises a therapeutically or prophylactically effective amount of another anti-HIV agent.

The invention also provides *in vitro* and *in vivo* assays for assessing the efficacy of therapeutics of the invention for treatment or prevention of infection with an immunodeficiency virus, in particular HIV infection.

The invention further relates to methods for treating or preventing immunodeficiency virus infection, in particular HIV, in mammals, including humans, by administering the therapeutic compositions of the invention. Methods of administration of the therapeutics of the invention for treatment or prevention of immunodeficiency virus infection are also provided.

The invention also provides methods for inhibiting the infection or replication of any isolate of an

$\gamma$  interferon inducible protein-10 ( $\gamma$ IP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B cell stimulatory factor (PBSF)); and/or a  $\beta$ (CC) chemokine or nucleic acid encoding a  $\beta$  chemokine selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), macrophage inflammatory protein-1 $\beta$  (MIP-1 $\beta$ ), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 $\gamma$  (MIP-1 $\gamma$ ), macrophage inflammatory protein-3 $\alpha$  (MIP-3 $\alpha$ ), macrophage inflammatory protein-3 $\beta$  (MIP-3 $\beta$ ), eotaxin, Exodus, and I-309; and/or the  $\gamma$ (C) chemokine, or nucleic acid encoding the  $\gamma$  chemokine, lymphotactin. In another embodiment, such compositions comprise a plurality of chemokines, e.g. at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 chemokines, selected from those listed above.

In one embodiment, the pharmaceutical compositions of the invention comprise 1, 2, 3, or 4 chemokines selected from among RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , or IL-8 or a therapeutically and prophylactically effective derivative or analogue thereof, in combination with one or more other chemokines, or a therapeutically and prophylactically effective derivative or analogue thereof. Such other chemokines are selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, lymphotactin and SDF-1. Pharmaceutical compositions comprising nucleic acids encoding such chemokines are also provided. In further embodiments, the pharmaceutical composition comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 chemokines, or nucleic acids encoding the same.

In another embodiment, the pharmaceutical composition comprises a  $\beta$  chemokine, or nucleic acid encoding a  $\beta$

and lymphotactin. Preferably, the chemokine has been purified. In another embodiment, the pharmaceutical composition further comprises a therapeutically or prophylactically effective amount of one or more chemokine, derivative or analogue thereof, selected from the group consisting of RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , IL-8, and SDF-1. Pharmaceutical compositions comprising nucleic acids encoding such chemokines, derivatives and analogues are also provided.

In another specific embodiment, the pharmaceutical composition comprises a therapeutically or prophylactically effective amount of a derivative or analogue of one or more of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin. Preferably, the chemokine derivative or analogue has been purified. In another embodiment, the pharmaceutical composition further comprises a therapeutically or prophylactically effective amount of one or more chemokine, derivative or analogue thereof, selected from the group consisting of RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , IL-8, and SDF-1. Pharmaceutical compositions comprising nucleic acids encoding such chemokines, derivatives and analogues listed above are also provided.

In another specific embodiment, the pharmaceutical composition comprises a therapeutically or prophylactically effective amount of one or more of MCP-2, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin. Preferably, the chemokine has been purified. In another embodiment, such pharmaceutical composition further comprises a therapeutically or prophylactically effective amount of one or more chemokine, derivative or analogue selected from the group consisting of RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , MCP-1, MCP-3, IL-8, and SDF-1. Pharmaceutical compositions comprising nucleic acids encoding such chemokines, derivatives or analogues are also provided.

In another specific embodiment, the pharmaceutical composition comprises a therapeutically or prophylactically

antigen. Preferably, the pharmaceutical composition comprises a chemokine, nucleic acid encoding a chemokine, or derivative or analogue thereof that binds separately to an  $\alpha$  and  $\beta$  chemokine receptor. In another preferred embodiment, the pharmaceutical composition comprises a chemokine, derivative or analogue that binds separately to a plurality of  $\alpha$  and/or  $\beta$  chemokine receptors. In a most preferred embodiment, the pharmaceutical composition comprises a chemokine, or a derivative or analogue thereof, that binds separately to both CXC CKR4 and CC CKR-5. In further embodiments, the chemokine, derivative and/or analogue binds separately to 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 chemokine receptors. In another preferred embodiment, the derivative or analogue of the invention is capable of binding separately to at least 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 chemokine receptors selected from the group consisting of CC CKR-1, CC CKR-2a, CC CKR-2b, CC CKR-3, CC CKR-4, CC CKR-5, CXC CKR4, IL-8RA, IL-8RB, Mig receptor,  $\gamma$ IP-10 receptor, and Duffy antigen. Pharmaceutical compositions containing nucleic acids encoding such chemokines, derivatives and/or analogues are also provided.

In a specific embodiment, the invention relates to RANTES and SDF-1 derivatives and analogues, or nucleic acids encoding RANTES and SDF-1 derivatives and analogues, that comprise, or alternatively consist of an amino acid sequence capable of binding to a chemokine receptor. In a preferred embodiment, the chemokine derivative or analogue is a molecule that comprises the amino acid sequence Lys-Asn-Asn-Asn-Arg-Gln-Val (SEQ ID NO:1) (amino acids 45-51 of mature SDF-1 (SWISS-PROT:P48061, June 1, 1996)), which is believed by the Inventors to bind the CXC CKR4 receptor, with the proviso that the molecule is less than 61, 60, 55, 50, 45, 40, 35, 30, 20, 15, 14, 13, 12, 11, 10, 9, 8, or 7 amino acids. In a particular embodiment, the chemokine derivative or analogue is a molecule that comprises the amino acid sequence Cys-Ala-Leu-Gln-Ile-Val-Ala-Arg-Leu-Lys-Asn-Asn-Asn-Arg-Gln-Val-Cys (SEQ ID NO:2, amino acids 36-52 of mature

fragments of SDF-1 comprising amino acids 45-49 or 34-50 of mature RANTES are provided, as well as such fragments with conservative substitutions. In specific embodiments described *infra*, RANTES and/or SDF-1 derivatives or analogues 5 comprising the amino acid sequences listed above are joined at amino or carboxy-termini via a peptide bond to an amino acid sequence of a different protein to form a chimeric, or fusion protein.

In a specific embodiment of the invention, proteins 10 consisting of or comprising a fragment of a chemokine consisting of at least 5 (continuous) amino acids of the chemokine is provided. In other embodiments, the fragment consists of at least 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70 or 80 amino acids of the chemokine. In specific 15 embodiments, such fragments are not larger than 10, 20, 30, 40, 50, 60, 70 or 80 amino acids. Derivatives or analogues of a chemokine include but are not limited to those molecules exhibiting antiviral activity and that comprise regions that are substantially homologous to a chemokine or fragment 20 thereof (e.g., in various embodiments, at least 60% or 70% or 80% or 90% or 95% identity over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art) or whose encoding nucleic acid is capable 25 of hybridizing to a coding chemokine sequence, under high stringency, moderately high stringency, or low stringency conditions. In a specific embodiment, the chemokine derivative retains the antigenicity (ability to bind to an anti-chemokine antibody) or immunogenicity of the chemokine. 30 Fragments and other derivatives of a chemokine that retain the ability to bind to a chemokine receptor are preferred.

By way of example and not limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): 35 Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500

high stringency screening are known in the art. For further guidance regarding hybridization conditions see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 5 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

The invention also relates to chemokine derivatives or analogues made by altering the chemokine sequence by substitutions, additions or deletions that provide for 10 molecules with anti-viral activity (e.g., inhibit infection or replication of an immunodeficiency virus, preferably HIV) or demonstrate the ability to bind to a chemokine receptor. Thus, the chemokine derivatives include polypeptides containing, as a primary amino acid sequence, all or part of 15 the chemokine amino acid sequence including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a polypeptide which is functionally active. For example, one or more amino acid residues within the sequence can be 20 substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the 25 nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids 30 include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such chemokine derivatives can be made either by chemical peptide synthesis or by recombinant production from nucleic acid encoding the chemokine which nucleic acid 35 has been mutated. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, in vitro site-directed mutagenesis (Hutchinson,

ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art.

5 Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. In a specific embodiment, a chimeric nucleic acid encoding a chemokine with a heterologous signal sequence is expressed such that the chimeric protein is expressed and processed by  
10 the cell to the mature chemokine.

In a preferred embodiment, the chimeric of the invention comprises the amino acid sequence Lys-Asn-Asn-Asn-Arg-Gln-Val (SEQ ID NO:1) or Cys-Ala-Leu-Gln-Ile-Val-Ala-Arg-Leu-Lys-Asn-Asn-Asn-Arg-Gln-Val-Cys (SEQ ID NO:2) of mature SDF-1 joined  
15 at its amino- and/or carboxy-terminus via a peptide bond to a different protein. In another preferred embodiment, the chimeric comprises the amino acid sequence Lys-Asn-Arg-Gln-Val (SEQ ID NO:3) or Cys-Ser-Asn-Pro-Ala-Val-Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys (SEQ ID NO:4) of mature RANTES  
20 (Schall, 1991, Cytokine 3:165-183) joined at its amino- and/or carboxy-terminus via a peptide bond to a different protein.

In other specific embodiments, the chimeric of the invention comprises a RANTES derivative in which the amino  
25 acid sequence Lys-Asn-Asn-Asn-Arg-Gln-Val (SEQ ID NO:1) or Lys-Asn-X-Arg-Gln-Val (SEQ ID NO:5) is substituted for the sequence Lys-Asn-Arg-Gln-Val (SEQ ID NO:3) in RANTES, or the amino acid sequence Cys-Ala-Leu-Gln-Ile-Val-Ala-Arg-Leu-Lys-Asn-Asn-Asn-Arg-Gln-Val-Cys (SEQ ID NO:2) is substituted for  
30 the sequence Cys-Ser-Asn-Pro-Ala-Val-Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys (SEQ ID NO:4) in RANTES. In other specific embodiments, the chimeric of the invention comprises a SDF-1 derivative in which the amino acid sequence Lys-Asn-Arg-Gln-Val (SEQ ID NO:3) or Lys-Asn-X-Arg-Gln-Val (SEQ ID  
35 NO:5) is substituted for the sequence Lys-Asn-Asn-Asn-Arg-Gln-Val (SEQ ID NO:1) in SDF-1, or the amino acid sequence Cys-Ser-Asn-Pro-Ala-Val-Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-

ID NO:4), wherein said RANTES fragment is less than 55 amino acids in length. Optionally, one or both of the fragment components of this chimeric are capable of binding one or more chemokine receptors. Molecules comprising or consisting of this chimeric sequence are provided, as are nucleic acids encoding the same.

Chimeric chemokines of the invention may be synthetic peptide fragments or full length synthetic chemokines in which are inserted specific sequences from  $\alpha$ ,  $\beta$ , and/or  $\gamma$  chemokines which optionally bind a chemokine receptor or inhibit immunodeficiency virus infection or replication, so as to bind one or more chemokine receptors. The primary sequence of the chemokines may also be used to predict tertiary structure of the molecules using computer simulation (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828); chemokine/chemokine chimeric recombinant genes can be designed in light of correlations between tertiary structure and biological function.

Derivatives mentioned above also can be cyclized, e.g., as described in Section 4.1.1.

Antiviral activity of the chemokines, nucleic acids encoding chemokines, or derivatives (including fragments and chimeric proteins) or analogues thereof, for treatment or prevention of HIV infection can be demonstrated by any of the methods disclosed in Section 4.2, 5, 6, 7, 8 and 9, *infra* or known to one skilled in the art.

#### 4.1.1. PREPARATION OF CHEMOKINES, DERIVATIVES AND ANALOGUES

The chemokines, derivatives or analogues of the invention can be obtained commercially or alternatively, purified from biological tissue or cell culture, or produced by recombinant or synthetic techniques known in the art.

Native chemokine preparations can be obtained from a variety of sources. Recombinant RANTES, MIP-1 $\alpha$  and MIP-1 $\beta$  are commercially available (Sigma Immunochemicals, St. Louis, MO; R&D Systems, Minneapolis, MN; and PeproTech, Rocky Hills,

183:2379-2384; Baggiolini et al., 1994, Advances in Immunology 55:97-179; Miller and Krangel, 1992, Immunology 12:17-46; and Schall, 1991, Cytokine 3:165-183) and can be isolated using well-known techniques in the art, such as  
5 screening a library, chemical synthesis, or polymerase chain reaction (PCR). Other chemokines may be cloned using routine recombinant techniques known in the art in combination with assays which select for known biochemical properties of the chemokine of interest. Cloned chemokine gene sequence can be  
10 modified by any of numerous strategies known in the art.

To recombinantly produce a chemokine, derivative or analogue, a nucleic acid sequence encoding the chemokine derivative or analogue is operatively linked to a promoter such that the chemokine, derivative, or analogue is produced  
15 from said sequence. For example, a vector can be introduced into a cell, within which cell the vector or a portion thereof is expressed, producing a chemokine or a portion thereof. In a preferred embodiment, the nucleic acid is DNA if the source of RNA polymerase is DNA-directed RNA  
20 polymerase, but the nucleic acid may also be RNA if the source of polymerase is RNA-directed RNA polymerase or if reverse transcriptase is present in the cell or provided to produce DNA from the RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be  
25 transcribed to produce the desired RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art.

A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are  
30 not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The  
35 expression elements of vectors vary in their strengths and specificities and depending on the host-vector system

38:647-658; Adames et al., 1985, Nature 318:533-538;  
Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse  
mammary tumor virus control region which is active in  
testicular, breast, lymphoid and mast cells (Leder et al.,  
5 1986, Cell 45:485-495), albumin gene control region which is  
active in liver (Pinkert et al., 1987, Genes and Devel.  
1:268-276), alpha-fetoprotein gene control region which is  
active in liver (Krumlauf et al., 1985, Mol. Cell. Biol.  
5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-  
10 antitrypsin gene control region which is active in the liver  
(Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-  
globin gene control region which is active in myeloid cells  
(Mogram et al., 1985, Nature 315:338-340; Kollias et al.,  
1986, Cell 46:89-94; myelin basic protein gene control region  
15 which is active in oligodendrocyte cells in the brain  
(Readhead et al., 1987, Cell 48:703-712); myosin light chain-  
2 gene control region which is active in skeletal muscle  
(Sani, 1985, Nature 314:283-286), and gonadotropic releasing  
hormone gene control region which is active in the  
20 hypothalamus (Mason et al., 1986, Science 234:1372-1378).  
The promoter element which is operatively linked to the  
nucleic acid encoding chemokine, derivative or analogue can  
also be a bacteriophage promoter with the source of the  
bacteriophage RNA polymerase expressed from a gene for the  
25 RNA polymerase on a separate plasmid, e.g., under the control  
of an inducible promoter, for example, the nucleic acid  
encoding chemokine, derivative, or analogue, operatively  
linked to the T7 RNA polymerase promoter with a separate  
plasmid encoding the T7 RNA polymerase.  
30 In addition, a host cell strain may be chosen which  
modulates the expression of the inserted sequences, or  
modifies and processes the gene product in the specific  
fashion desired. Expression from certain promoters can be  
elevated in the presence of certain inducers; thus,  
35 expression of the genetically engineered chemokine,  
derivative or analogue may be controlled. Furthermore,  
different host cells have characteristic and specific

kilobases) are unstable in those vectors. Protocols are known to one skilled in the art and kits for site-directed mutagenesis are widely available from biotechnology supply companies, for example from Amersham Life Science, Inc. 5 (Arlington Heights, IL) and Stratagene Cloning Systems (La Jolla, CA).

In other specific embodiments, the chemokine derivative or analogue may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analogue, or 10 derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding 15 frame, and expressing the chimeric product by methods commonly known in the art.

In addition, chemokines, derivatives (including fragments and chimeric proteins), and analogues can be chemically synthesized. See, e.g., Clark-Lewis et al., 1991, 20 *Biochem.* 30:3128-3135 and Merrifield, 1963, *J. Amer. Chem. Soc.* 85:2149-2156. For example, chemokines, derivatives and analogues can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., see Creighton, 1983, 25 *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 50-60). Chemokines, derivatives and analogues can also be synthesized by use of a peptide synthesizer. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the 30 Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49). Furthermore, if desired, nonclassical amino acids or chemical amino acid analogues can be introduced as a substitution or addition into the chemokine, 35 derivative or analogue. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-

or predicted inclusion within a helical formation during synthesis. For these situations, the above cycle can be modified by repeating step 4 a second time immediately upon completion of the first 45 minute coupling step to "double couple" the amino acid of interest. Additionally, in the first coupling step in polypeptide synthesis, the resin can be allowed to swell for more efficient coupling by increasing the time of mixing in the initial DMF washes to three 15 minute washes rather than three 30 second washes. After polypeptide synthesis, the peptide can be cleaved from the resin as follows: (1) washing the polypeptide-resin three times for 30 seconds with DMF; (2) removing the Fmoc protective group on the amino terminus by washing two times for 15 minutes in 20% piperidine in DMF; (3) washing the polypeptide-resin three times for 30 seconds with DMF; and (4) mixing a cleavage cocktail consisting of 95% trifluoroacetic acid (TFA), 2.4% water, 2.4% phenol, and 0.2% triisopropylsilane with the polypeptide-resin for two hours, then filtering the peptide in the cleavage cocktail away from the resin, and precipitating the peptide out of solution by addition of two volumes of ethyl ether. To isolate the polypeptide, the ether-peptide solution can be allowed to sit at -20°C for 20 minutes, then centrifuged at 6,000xG for 5 minutes to pellet the polypeptide, and the polypeptide can be washed three times with ethyl ether to remove residual cleavage cocktail ingredients. The final polypeptide product can be purified by reversed phase high pressure liquid chromatography (RP-HPLC) with the primary solvent consisting of 0.1% TFA and the eluting buffer consisting of 80% acetonitrile and 0.1% TFA. The purified polypeptide can then be lyophilized to a powder.

In one embodiment, the polypeptide is a cyclic peptide. Cyclized polypeptides can be prepared by any method known in the art. For example, but not by way of limitation, disulfide bridge formation can be achieved by (1) dissolving the purified peptide at a concentration of between 0.1-0.5 mg/ml in 0.01 M ammonium acetate, pH 7.5; (2) adding 0.01 M

protected amino acid, an allyl protected amino acid can be introduced into the sequence of the polypeptide, at the amino-terminus, carboxy-terminus or internally, such that the polypeptide can be cyclized.

5 In another embodiment, a polypeptide that is a chemokine or derivative thereof is synthesized to contain disulfide bridges corresponding to those observed in the native chemokine. Techniques known in the art may be applied to achieve selective disulfide bridge formation, including but  
10 not limited to the use of cysteine residues that having different protection groups. For example, full length RANTES may be synthesized as described above, but incorporating acetamidomethyl (AcM) protected cysteine amino acids at positions 10 and 37 of the polypeptide. After synthesis is  
15 completed, the polypeptide is cleaved off the support resin. The cleavage, deprotection reaction is for 2 hours in 88% trifluoroacetic acid, 5% water, 5% phenol, and 2% triisopropylsilane. This step additionally deprotects the initial trityl groups from Cys11 and Cys50. The polypeptides  
20 are purified by reverse phase HPLC to >95% purity. The polypeptide is diluted in 0.1M NaOH, pH 8.0 at a concentration of 0.25 mg/ml and allowed to mix 24 hours at room temperature to create a disulfide bridge between amino acids Cys11 and Cys50. Disulfide linked polypeptide is  
25 repurified by reverse phase HPLC. The polypeptide is dissolved in 80% acetic acid at a concentration of 0.25 mg/ml and solid iodine is added at a concentration of 0.25 mg/ml and allowed to mix for 24 hours at room temperature. This step deprotects the AcM groups from Cys10 and Cys34 amino  
30 acids to form the final disulfide bridge. An equal volume of water is added to the mixture which is extracted four times with 50 mls of CCl<sub>4</sub> to remove the iodine. The product, now containing disulfide bridges between amino acids Cys11 and Cys 50 and between amino acids Cys10 and Cys34, is  
35 lyophilized.

The chemokines, derivatives, or analogues of the invention may be synthesized in their entirety by the

#### 4.2. ASSAYS FOR RECEPTOR BINDING AND INHIBITION OF VIRAL INFECTION OR REPLICATION BY CHEMOKINE PROTEINS, DERIVATIVES AND ANALOGUES

The ability of chemokines or the derivatives or analogues thereof to bind chemokine receptors and thereby  
5 interfere with viral infection or replication can be assayed by various methods.

In a preferred embodiment, the chemokine derivatives (including fragments and chimeric proteins) or analogues, bind protein sequences contained in the extracellular domain  
10 of a chemokine receptor. Binding can be assayed by means well-known in the art. For example, bioassays may be performed in which cells known to be expressing a chemokine receptor are exposed to the chemokine derivative or analogue to be tested and assayed for a known effect (e.g., signal  
15 transduction). Alternatively, chemokines, derivatives or analogues can be tested for the ability to bind chemokine receptors by procedures, including but not limited to, protein affinity chromatography, affinity blotting, immunoprecipitation, cross-linking, and library based methods  
20 such as protein probing, phage display, and the two-hybrid system (see, generally, Phizicky et al., 1995, Microbiol. Rev. 59:94-123). Further, where DNA encoding a chemokine receptor has been identified, this sequence may be routinely manipulated in known assays to identify chemokine derivatives  
25 or analogues which bind to the extracellular domain of the receptor. Such assays include, but are limited to, in vitro cell aggregation and interaction trap assays. Nucleic acids encoding CC CKR-1 (Neote et al., 1993, Cell 72:415-425); CC CKR-2A and CC CKR-2B (Chavo et al., 1994, Proc. Natl. Acad.  
30 Sci. 91:2752-2756); CC CKR-3 (Daugherty et al., 1996, J. Exp. Med. 183:2349-2354 and Ponath et al., 1996, J. Exp. Med. 183:1-12); CC CKR-4 (Power et al., 1995, J. Biol. Chem. 270:19495-19500); CC CKR-5 (Samson et al., 1996, Biochemistry 35:3362-3367); CXC CKR4 (Feng et al., 1996, Science 272:872-  
35 877); IL-8RA and IL-8RB (Kunz et al., 1991, J. Biol. Chem. 267:9101-9106 and Gerard et al., 1994, Corr. Opin. Immunol.

a range of concentrations may be tested. This range should include a control culture wherein no chemokine, derivative and/or analogue has been added. Standard conditions for culturing, well known to those of ordinary skill in the art, 5 are used. After incubation for an appropriate period, such as, for example, 24 hours at 37°C, the culture is examined microscopically for the presence of multinucleated giant cells, which are indicative of cell fusion and syncytia formation.

10 In one embodiment, an *in vitro* cell-free infectivity assay is performed using primary macrophages and the macrophage-tropic isolate HIV-1<sub>mac</sub>, the first described macrophage-tropic HIV-1 isolate (see, Gartner et al., 1986, Science 233:215). According to this assay, primary 15 macrophage cells isolated according to methods known in the art are infected with HIV-1<sub>mac</sub> that has been propagated and maintained only in primary macrophages. The input immunodeficiency virus is incubated with primary macrophages in the presence of concentrations of the chemokine, 20 derivative, or analogue to be tested. After a defined period of infection, unbound virus is removed by washing, and the cells are placed in culture. The level of virus replication in this assay may be assessed by techniques known in the art, including but not limited to, measuring reverse transcriptase 25 (RT) levels, or the release of extracellular p24 core antigen at different days post-infection. A constant level of inhibition of viral infection or replication is determined by measuring output HIV p24 levels (or another indicator of viral infection or replication, such as for example, RT) 30 relative to control assays performed in the absence of the chemokine, derivative or analogue. Preferably, the chemokine derivative or analogue reduces levels of virus, as measured by, for example, p24, by  $\geq 50\%$  relative to control assays carried out in the absence of test compound. The presence of 35 p24 may be determined using methods known in the art, such as commercially available enzyme-linked immunosorbent assays (Coulter, Hialeah, Florida; Abbott Laboratories, Hvalstad,

As above, the input immunodeficiency virus is incubated with target cells in the presence of various quantities of the test chemokine, derivative, or analogue to be tested. After a defined period of infection, unbound virus is removed  
5 by washing, and the cells are placed in culture. As above, the level of virus replication in this assay may be assessed by techniques known in the art, including but not limited to, measuring reverse transcriptase levels or the release of extracellular p24 core antigen at different days post-  
10 infection. A constant level of inhibition of viral infection or replication is determined by measuring output HIV p24 levels (or another indicator) relative to control assays performed in the absence of the chemokine, derivative or analogue. Preferably, the chemokine derivative or analogue  
15 reduces levels of virus, as measured by, for example, p24, by  $\geq 50\%$  relative to control assays carried out in the absence of test compound.

In addition to evaluating the antiviral activity of a chemokine, derivative or analogue, the primary CD4<sup>+</sup> PBMC/HIV-1  
20 assay may be used to formulate pharmaceutical compositions containing combinations of chemokines, derivatives and/or chemokine analogues, effective in inhibiting infection or replication of the viral isolates assayed and may be applied to formulate a pharmaceutical composition effective in  
25 inhibiting infection or replication of a plurality of T-tropic strains.

In an additional embodiment, an in vitro cell-free infectivity assay is performed using PM1 cells and HIV<sub>Mal</sub>. As above, the input immunodeficiency virus is incubated with  
30 target cells (PM1) in the presence of various quantities of the test chemokine, derivative, or analogue to be tested. After a defined period of infection, unbound virus is removed by washing, and the cells are placed in culture. As above, the level of immunodeficiency virus replication in this assay  
35 may be assessed by techniques known in the art, including but not limited to, measuring reverse transcriptase levels or the release of extracellular p24 core antigen at different days

derivatives and/or analogues to inhibit transmission of isolates specific to the patient at a given time (See Section 4.3).

In another embodiment, chemokine(s), derivative(s) and/or analogue(s) are identified by their ability to inhibit the isolation of primary immunodeficiency virus isolates from primary target cells removed from an infected individual. According to this embodiment, CD4<sup>+</sup> target cells isolated from an HIV<sup>+</sup> individual using techniques known in the art are exposed to one or more chemokines, derivatives, and/or analogues and known techniques, such as those described *infra*, are applied to isolate the virus from the cells. In a preferred embodiment, these chemokines, derivatives and/or analogues are known or indicated by the *in vitro* assays described herein to inhibit the infection or replication of one or more HIV-1 strains. Parallel control experiments are performed in which the same virus isolation technique is performed in the absence of chemokines, derivatives, and/or analogues. An inability or reduced ability to isolate immunodeficiency virus in the test samples, but not the control sample indicates that the primary immunodeficiency virus isolates are sensitive to the test chemokines, derivatives, and/or analogues.

The chemokine protein, derivative, or analogue compositions may then be combined with suitable pharmaceutically acceptable carriers and administered by techniques known in the art, such as those described in Section 4.7 *infra*.

Techniques known in the art may be applied to formulate compositions displaying minimal toxicity. For each *in vitro* test of chemokines, derivatives and/or analogues of the invention, it is important to determine the effects on cell proliferation and viability. Methods for assessing effects of the compounds tested on cell proliferation include, but are not limited to, assaying for thymidine uptake and counting cells (using, for example, a hemocytometer or flow cytometer). Methods for assessing cell viability include,

infra), as well as nucleic acids encoding such chemokines, derivatives and analogues thereof.

The invention also provides methods for treating or preventing viral infections, by administering an effective amount of a Therapeutic that binds to one or more chemokine receptors. In one embodiment, the Therapeutic is a chemokine, derivative, or analogue that binds to an  $\alpha$  and  $\beta$  chemokine receptor. In further embodiments, the Therapeutic is a chemokine, derivative or analogue that binds to 3, 4, 5, 6, 7, 8, 9, or 10 chemokine receptors. In a preferred embodiment, the Therapeutic is able to bind CC CKR-5 and CXC CKR4. In another embodiment, the pharmaceutical composition contains a plurality of Therapeutic chemokines, derivatives, and/or analogues.

The Therapeutics of the invention can also be tested in vivo for toxicity and/or the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal models including but not limited to rats, mice, chickens, cows, sheep, dogs, cats, monkeys, apes, rabbits, etc. For in vivo testing, prior to administration to humans, any animal model system known in the art may be used.

#### 4.3. FORMULATING A PATIENT-SPECIFIC PHARMACEUTICAL COMPOSITION

In particular embodiments, the invention provides methods for formulating a pharmaceutical composition which comprises the chemokines, derivatives, and/or analogues that inhibit replication and/or infection of immunodeficiency virus isolate(s) found in an individual at a given time and preferably does not comprise chemokines or derivatives and/or analogues thereof that do not inhibit replication and/or infection of such isolate(s). Such methods are achieved by isolating primary immunodeficiency virus(es) from PBMC's and/or lymph nodes of a patient, and testing the virus(es) against a panel of chemokines (or derivatives or analogues thereof) to determine which particular chemokines are active

immunodeficiency virus isolate of interest. In particular the pharmaceutical composition comprises 2, 3, 4, 5, 6, 7, 8, 9, or 10 chemokines, derivatives, and or analogues determined to have antiviral activity. Assays described *infra*, may be  
5 used to determine optimum relative concentrations of the chemokine, derivative, and/or analogue components of the pharmaceutical composition.

The invention therefore provides methods by which to identify chemokine(s), derivative(s), or analogue(s) that  
10 inhibit infection or replication of a specific isolate of an immunodeficiency virus, particularly an HIV-1 isolate, and by which pharmaceutical compositions containing these chemokines or therapeutically or prophylactically effective derivatives, or analogues, alone or in combination, are routinely  
15 formulated. The invention further provides methods for treating or preventing immunodeficiency virus infections, in particular HIV infection, in mammals, including humans, by administering the therapeutic compositions of the invention.

The invention thus provides methods for formulating on a  
20 patient-to-patient basis, a pharmaceutical composition comprising chemokines, derivatives and/or analogues that are known to be effective against isolate(s) of an immunodeficiency virus present in an individual at a given time.

25

#### 4.4. THERAPEUTIC USES

The invention provides for treatment or prevention of diseases and disorders associated with infection by an immunodeficiency virus, particularly, HIV, by administration  
30 of a Therapeutic. Such Therapeutics include, but are not limited to: chemokines and therapeutically and prophylactically effective chemokine derivatives and/or analogues, *i.e.*, those derivatives and analogues which prevent or treat HIV infection (*e.g.* as demonstrated *in vitro*  
35 assays described *infra*), as well as nucleic acids encoding such chemokines, derivatives and analogues thereof (*e.g.*, for use in gene therapy). Examples of Therapeutics are those

agent provided for treatment or prevention of HIV. In another embodiment, the Therapeutic is administered in combination with one or more anti-viral compounds, for example, protease inhibitors (e.g., zidovudine) and/or reverse transcriptase inhibitors (e.g., zidovudine (AZT), lamivudine (3TC), dideoxyinosine (ddI), dideoxycytidine (ddC)). The Therapeutic may also be administered in conjunction with chemotherapy (e.g., treatment with adriamycin, bleomycin, vincristine, vinblastine, doxorubicin and/or Taxol) or other therapies known in the art.

#### 4.4.1. GENE THERAPY

In a specific embodiment, nucleic acids comprising a sequence encoding chemokine, protein derivative or protein analogue, effective at inhibiting HIV replication and/or infection in vitro are administered for treatment or prevention of HIV infection, by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. In this embodiment of the invention, the nucleic acid produces its encoded protein that mediates a therapeutic effect by preventing or treating HIV infection. For example, any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, the nucleic acid encoding chemokine, derivative or analogue is part of an expression

expressing the receptors), etc. In a specific embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated April 16, 1992 (Wu et al.); WO 92/22635 dated December 23, 1992 (Wilson et al.); WO 92/20316 dated November 26, 1992 (Findeis et al.); WO 93/14188 dated July 22, 1993 (Clarke et al.), WO 93/20221 dated October 14, 1993 (Young)). In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand  
10 comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies,  
15 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

In a specific embodiment, a viral vector that contains the nucleic acid sequence encoding a chemokine, derivative or analogue is used. For example, a retroviral vector can be  
20 used (see Miller et al., 1993, Meth. Enzymol. 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome. Retroviral vectors are maintained in infected cells by integration into genomic sites upon cell  
25 division. The nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the  
30 *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, J. Clin. Invest. 93:644-651; Kiem et al., 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993,  
35 Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; 5 Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the 10 cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, recombinant blood cells (e.g., hematopoietic stem 15 or progenitor cells) are administered intravenously. Additionally, epithelial cells can be injected, e.g., subcutaneously, or recombinant skin cells (e.g., keratinocytes) may be applied as a skin graft onto the patient. The amount of cells envisioned for use depends on 20 the desired effect, patient state, etc., and can be determined by one skilled in the art.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid sequence coding for chemokine, or therapeutically or prophylactically effective derivative, 25 or analogue is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells, preferably hematopoietic stem or progenitor 30 cells, are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention.

#### 4.7. THERAPEUTIC/PROPHYLACTIC COMPOSITIONS AND METHODS OF ADMINISTERING

The invention provides methods of treatment and prevention by administration to a subject in which such treatment or prevention is desired a therapeutically or prophylactically effective amount of a Therapeutic of the invention. The subject is preferably an animal, including, but not limited to, animals such as monkeys, sheep, cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. The subject can be a fetus, child, or adult. In a preferred aspect, the Therapeutic is substantially purified.

Formulations and methods of administration that can be employed when the Therapeutic comprises a nucleic acid are described in Sections 4.1 and 4.4.1 above; additional appropriate formulations and routes of administration can be selected from among those described hereinbelow.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the Therapeutic, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the therapeutic compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an

Release, 1984, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida, vol. 2, pp. 115-138).

Other controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533).

5 In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered by gene therapy methods as described *supra* in Section 4.4.1.

The present invention also provides pharmaceutical  
10 compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a therapeutically acceptable carrier. In a specific embodiment, the term "therapeutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the  
15 U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the Therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water  
20 and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and  
25 glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium  
30 chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules,  
35 powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral

triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder  
5 or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vivo* and/or *in vitro* assays may optionally be employed to help predict optimal dosage ranges. The precise dose to be employed in the formulation will also  
10 depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 1-1000  
15 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test  
20 systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit  
25 comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or  
30 biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

relative to control assays carried out in the absence of test compound.

6. **EXAMPLE: PRIMARY CD4<sup>+</sup> PBMC/PRIMARY HIV-1 ISOLATE  
CELL FREE INFECTIVITY ASSAY FOR CHEMOKINE  
SUPPRESSION**

- 5 The following assay is used to determine the ability of a chemokine, derivative or analogue to interfere with the infection or replication of a primary HIV-1 isolate in primary CD4<sup>+</sup> cells. Target cells can either be peripheral
- 10 blood mononuclear cells (PBMC's) depleted of CD8<sup>+</sup> cells using anti-CD8 immunomagnetic beads or CD4<sup>+</sup> PBMC's purified with anti-CD4 immunomagnetic beads. Immunomagnetic bead depletion/purification protocols are carried out according to manufacturer's instructions (Dynal A.S., Norway).
- 15 Viruses are isolated according to procedures known in the art. Briefly, isolates are obtained by co-culturing of 1 to  $2 \times 10^6$  PBMC's from HIV-1 infected individuals with phytohemagglutinin (PHA)-stimulated PBMC from two HIV-1 negative blood donors. The cultures are maintained in
- 20 complete RPMI 1640 medium (Gibco) containing 10% fetal calf serum (FCS), 5U/ml of rIL2 (R & D Systems, Minneapolis, Minnesota), 2µg/ml polybrene (Sigma, St. Louis, Missouri) and antibiotics. Virus antigen production is measured in supernatants twice weekly using an HIV-1 p24 antigen capture
- 25 ELISA (Coulter, Hialeah, FL). Virus stocks are generated from the p24 antigen capture assay positive supernatants by passaging of the virus isolates once or twice in PHA stimulated blood donor PBMC's. The virus containing supernatants are aliquotted and cryopreserved at -75°C.
- 30 The primary isolates are titered before use so that known doses can be assayed. To determine the 50% tissue culture infectious dose (TCID<sub>50</sub>) of virus stocks, the PBMC's from one donor are activated with PHA and cultured for three days in complete medium of RPMI. The activated PBMC's are
- 35 thereafter aliquotted in fetal calf serum containing 10% DMSO and cryopreserved at -15°C until use. At the time of virus

Reduced levels of virus in test samples as indicated by reduced levels of p24 in the ELISA relative to the controls indicate that the chemokine, derivative or analogue interferes with the infection of the primary HIV isolate in 5 primary CD4<sup>+</sup> cells.

7. **EXAMPLE: PM1/HIV-1<sub>BAL</sub> CELL FREE  
INFECTIVITY ASSAY FOR CHEMOKINE SUPPRESSION**

The following assay is used to determine the ability of  
10 a chemokine, derivative or analogue to interfere with the  
infection or replication of HIV-1<sub>BAL</sub> in the CD4<sup>+</sup> T-cell clone  
(PM1) which is susceptible to both primary and macrophage-  
tropic HIV-1 isolates. The PM1/HIV-1<sub>BAL</sub> test system is  
standardized in 48-well microliter plates using PM1 cells  
15 (available from the NIH AIDS Research and Reference Reagent  
Program) acutely infected with HIV-1<sub>BAL</sub>. PM1 cells  
(2x10<sup>5</sup>/test) are infected with HIV-1<sub>BAL</sub> (10-50 TCID<sub>50</sub>/1x10<sup>6</sup>  
cells) for 2 hr at 37°C, then washed three times with pre-  
warmed phosphate buffered saline (PBS) and resuspended in  
20 complete culture medium (250 µl per test) containing  
different dilutions of the chemokine, derivative and/or  
analogue composition to be assayed. At least four untreated  
controls, resuspended in complete medium, with or without  
exogenous interleukin-2 (IL-2), are always handled in  
25 parallel to treated cultures. The controls do not contain  
chemokine, derivative or analogue. At day 3 post-infection,  
250 µl of fresh chemokine, derivative or analogue composition  
containing the same original concentration of the respective  
test composition is added to each culture. The level of  
30 virus replication is assessed by measuring the release of  
extracellular p24 core antigen at different days post-  
infection. Five to nine days postinfection, the cultures are  
harvested, centrifuged to remove the cells and tested for  
HIV-1 p24 antigen by a commercial ELISA test (Coulter,  
35 Hialeah, FL).

Reduced levels of virus in test samples as indicated by  
reduced levels of p24 in the ELISA relative to the controls

relative to control assays carried out in the absence of test compound.

5           9.     **EXAMPLE: ASSAY FOR THE EFFECT OF  
                  COMPOSITIONS OF THE INVENTION ON  
                  CELLULAR PROLIFERATION AND VIABILITY**

          To rule out the possibility that the antiviral activity  
of the compositions assayed in Sections 5, 6, 7, and 8 may be  
due to a negative effect on cellular viability or  
proliferation, the effect of these compositions on the  
10 proliferative response and viability of the target cells is  
determined for every in vitro test. For example, the effect  
of the chemokine, derivative, or analogue tested in the  
primary CD4<sup>+</sup> PBMC/primary HIV-1<sub><sub>84</sub></sub> isolate cell-free  
infectivity assay (Section 7) on the proliferative response  
15 of primary CD4<sup>+</sup> PBMC may be determined. Peripheral blood  
mononuclear cells are separated by Ficoll gradient  
centrifugation and placed in round-bottom 96-well plates  
(10<sup>5</sup> cells/well). [<sup>3</sup>H]-Thymidine incorporation by stimulated  
cells is monitored in the presence of concentrations of the  
20 compositions corresponding to that used in the in vitro  
suppression assay (Section 7) and compared with [<sup>3</sup>H]-Thymidine  
incorporation in controls that have not been treated with the  
test composition. The test sample average corrected counts  
per minute from triplicate cultures and the percent  
25 radionucleotide incorporation is compared with that observed  
for the control. Comparable levels of [<sup>3</sup>H]-Thymidine  
incorporation in the test and control samples is indicative  
that antiviral activity observed in the cell free infectivity  
assay is not due to the suppression of cellular  
30 proliferation.

          The effect of the chemokine, derivative, or analogue  
tested on the viability of primary CD4<sup>+</sup> PBMC is determined  
applying techniques known in the art using trypan blue dye  
exclusion.

35           The present invention is not to be limited in scope by  
the specific embodiments described herein. Indeed, various

**WHAT IS CLAIMED IS:**

1. A method of identifying one or more chemokines for use in a pharmaceutical composition having anti-HIV activity against one or more HIV-1 isolates present in an individual  
5 at a given time comprising contacting a first aliquot of HIV cells obtained from said individual with a chemokine or derivative or analogue thereof; and comparing the ability to isolate HIV from said cells with the ability to isolate HIV from a second aliquot of HIV cells obtained from said  
10 individual that are not contacted with said chemokine, derivative or analogue, wherein a decrease in the ability to isolate virus in the presence of said chemokine, derivative or analogue is indicative that the chemokine, derivative or analogue has anti-viral activity against said HIV-1 isolates.  
15
2. The method of claim 1, further comprising the step of combining in a composition more than one of said chemokine, derivative or analogue demonstrating anti-viral activity against said HIV-1 isolates.  
20
3. The method of claim 2 in which at least 3 of said chemokine, derivative or analogue are combined.
4. The method of claim 1 which further comprises  
25 repeating said contacting and comparing steps for at least 3 individual cytokines, derivatives or analogues.
5. The method of claim 1 which further comprises repeating said contacting and comparing steps for at least 5  
30 individual cytokines, derivatives or analogues.
6. The method of claim 4 or 5 in which the chemokines, derivatives, or analogues are selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ ,  
35 MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin.

12. The method of claim 9 in which the primary isolate is syncytia inducing.

13. The method of claim 9 in which the primary isolate 5 is non-syncytia inducing.

14. The method of claim 8 in which said assaying of the chemokine, derivative or analogue is by a method comprising:

- (a) measuring HIV-1 levels in cultures of HIV 10 cells obtained from the patient which have been contacted with the chemokine, derivative or analogue; and
- (b) comparing said measured HIV-1 levels with said levels in said cells not so contacted with the chemokine, derivative or analogue, wherein a lower HIV-1 level in 15 cultures of said contacted cells indicates that the chemokine, derivative or analogue has anti-HIV activity.

15. The method of claim 14 which further comprises repeating steps (a) and (b) for at least 3 individual 20 chemokines, or derivatives or analogues.

16. The method of claim 14 which further comprises repeating steps (a) and (b) for at least 5 individual chemokines, or derivatives or analogues.

25

17. The method of claim 15 or 16 in which the chemokines, derivatives, or analogues are selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- 30  $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin.

18. A method of treating or preventing HIV infection or replication in a human subject comprising administering to the subject in which such treatment or prevention is desired 35 a pharmaceutical composition comprising a chemokine selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-

25. A method of treating or preventing HIV infection or replication in a human subject comprising administering to the subject in which such treatment or prevention is desired an amount of a purified protein effective to treat or prevent  
5 HIV infection in which the protein comprises a fragment or derivative of a chemokine selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin.

10

26. The method of claim 25 in which the only chemokine fragments or derivatives in said composition are those demonstrated to have activity against a primary HIV isolate from said subject.

15

27. The method of claim 25 in which fragments or derivatives of at least 3 different ones of said chemokines are administered to the subject.

20

28. The method of claim 25 which further comprises administering to the subject an anti-viral drug other than a chemokine, in an amount effective to inhibit HIV infection or replication.

25

29. The method of claim 28 in which the other anti-viral drug is selected from one or more of the group consisting of AZT, ddI, ddC, 3TC, and zidovudine.

30. The method of claim 25 in which the protein is  
30 administered intramuscularly.

31. A method of treating or preventing HIV infection or replication in a human subject comprising administering to the subject in which such treatment or prevention is desired  
35 an amount of a nucleic acid effective to treat or prevent HIV infection, in which the nucleic acid encodes a fragment or derivative of a chemokine selected from the group consisting

eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, lymphotactin and SDF-1; together in an amount effective to inhibit HIV infection or replication.

5        38. A pharmaceutical composition comprising a chemokine selected from the group consisting of MCP-2, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin, in an amount effective to inhibit HIV infection or replication; and  
10 a pharmaceutically acceptable carrier.

39. The pharmaceutical composition of claim 38 wherein the chemokine is purified.

15        40. The pharmaceutical composition of claim 38 further comprising at least 1, 2, 3, 4, 5, 6, 8, or 9 chemokines in an amount effective to inhibit HIV infection or replication.

41. The pharmaceutical composition of claim 38 further  
20 comprising RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , MCP-1, MCP-3, IL-8 or SDF-1 together in an amount effective to inhibit HIV infection or replication.

42. The pharmaceutical composition of claim 41 wherein  
25 the chemokines are purified.

43. A pharmaceutical composition comprising a derivative or analogue of a chemokine selected from the group consisting of MCP-2, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin,  
30 Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin, in an amount effective to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

35        44. The pharmaceutical composition of claim 43 wherein the chemokine derivative or analogue is purified.

51. A pharmaceutical composition comprising a chemokine derivative or analogue that binds separately to a plurality of chemokine receptors selected from the group consisting of CC CKR-1, CC CKR-2a, CC CKR-2b, CC CKR-3, CC CKR-4, CC CKR-5, 5 CxC CKR4, IL-8RA, IL-8RB, Mig receptor,  $\gamma$ IP-10 receptor and Duffy antigen, in an amount effective to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

10 52. The pharmaceutical composition of claim 51, wherein the derivative or analogue binds separately to CC CKR-5 and CxC CKR4.

53. The method of claim 8 in which the chemokine, 15 derivative or analogue is purified.

54. The method of claim 18 in which the chemokine is purified.

20 55. The method of claim 21 or 32 in which the chemokines are purified.

56. A purified fragment of a RANTES protein comprising the amino acid sequence Lys-Asn-Arg-Gln-Val (SEQ ID NO:3), 25 with the proviso that the fragment is less than 55 amino acids in length.

57. A purified derivative or analogue of the fragment of claim 56 that has only conservative substitutions in 30 sequence relative to the fragment.

58. A purified fragment of a RANTES protein comprising the amino acid sequence Cys-Ser-Asn-Pro-Ala-Val-Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys (SEQ ID NO:4), with the 35 proviso that the fragment is less than 55 amino acids in length.

the subject in which such treatment or prevention is desired the pharmaceutical composition of claim 65.

67. A chimeric protein comprising a fragment of RANTES  
5 comprising the amino acid sequence Cys-Ser-Asn-Pro-Ala-Val-  
Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys (SEQ ID NO:4)  
wherein said fragment is less than 55 amino acids in length  
and is capable of binding a chemokine receptor; fused via a  
covalent bond to an amino acid sequence of a molecule other  
10 than RANTES.

68. A pharmaceutical composition comprising the  
chimeric protein of claim 67 in an amount effective to  
inhibit HIV infection or replication; and a pharmaceutically  
15 acceptable carrier.

69. A method of treating or preventing HIV infection or  
replication in a human subject comprising administering to  
the subject in which such treatment or prevention is desired  
20 the pharmaceutical composition of claim 68.

70. A chimeric protein comprising a fragment of SDF-1  
comprising the amino acid sequence Lys-Asn-Asn-Asn-Arg-Gln-  
Val(SEQ ID NO:1), wherein said fragment is less than 60 amino  
25 acids in length and is capable of binding a chemokine  
receptor; fused via a covalent bond to an amino acid sequence  
of a molecule other than SDF-1.

71. A chimeric protein comprising a fragment of SDF-1  
30 comprising the amino acid sequence Cys-Ala-Leu-Gln-Ile-Val-  
Ala-Arg-Leu-Lys-Asn-Asn-Asn-Arg-Gln-Val-Cys (SEQ ID NO:2)  
wherein said fragment is less than 60 amino acids in length  
and is capable of binding a chemokine receptor; fused via a  
covalent bond to an amino acid sequence of a molecule other  
35 than SDF-1.

inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

80. A method of treating or preventing HIV infection or replication in a human subject comprising administering to the subject in which such treatment or prevention is desired the pharmaceutical composition of claim 79.

81. A chimeric protein comprising a RANTES derivative wherein the amino acid sequence Cys-Ala-Leu-Gln-Ile-Val-Ala-Arg-Leu-Lys-Asn-Asn-Asn-Arg-Gln-Val-Cys (SEQ ID NO:2) is substituted for the sequence Cys-Ser-Asn-Pro-Ala-Val-Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys (SEQ ID NO:4) in RANTES.

82. A pharmaceutical composition comprising the chimeric protein of claim 81 in an amount effective to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

83. A method of treating or preventing HIV infection or replication in a human subject comprising administering to the subject in which such treatment or prevention is desired the pharmaceutical composition of claim 82.

84. A chimeric protein comprising a SDF-1 derivative wherein the amino acid sequence Lys-Asn-Arg-Gln-Val (SEQ ID NO:3) or Lys-Asn-X-Arg-Gln-Val (SEQ ID NO:5) is substituted for the sequence Lys-Asn-Asn-Asn-Arg-Gln-Val (SEQ ID NO:1) in SDF-1.

85. A pharmaceutical composition comprising the chimeric protein of claim 84 in an amount effective to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

86. A method of treating or preventing HIV infection or replication in a human subject comprising administering to

presence of said chemokine, derivative or analogue is indicative that the chemokine, derivative or analogue has anti-viral activity against said HIV-1 isolates.

5        93. The method of claim 92, further comprising the step of combining in a composition more than one of said chemokine, derivative or analogue demonstrating anti-viral activity against said HIV-1 isolates.

10       94. The method of claim 93 in which at least 3 of said chemokine, derivative or analogue are combined.

95. The method of claim 92 which further comprises repeating said contacting and comparing steps for at least 3  
15 individual cytokines, derivatives or analogues.

96. The method of claim 92 which further comprises repeating said contacting and comparing steps for at least 5  
individual cytokines, derivatives or analogues.

20

97. The method of claim 95 or 96 in which the chemokines, derivatives, or analogues are selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, PF4, NAP-2, GRO-  
25  $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2, and lymphotactin.

98. A purified derivative or analogue of a chemokine selected from the group consisting of MCP-2, MCP-4, MIP-1 $\gamma$ , MIP-3 $\alpha$ , MIP-3 $\beta$ , eotaxin, Exodus, I-309,  $\gamma$ IP-10, IL-8, PF4,  
30 NAP-2, GRO- $\alpha$ , GRO- $\beta$ , GRO- $\gamma$ , ENA-78, GCP-2 and lymphotactin, which derivative inhibits HIV infection or replication.

99. The derivative or analogue of claim 98 that has only conservative substitutions in sequence relative to the  
35 chemokine.

isoleucine residues at amino acid numbers 28 and 30, respectively.

108. A pharmaceutical composition comprising the derivative of claim 107; and a pharmaceutically acceptable carrier.

109. A chimeric protein comprising a fragment of SDF-1 comprising the amino acid sequence Lys-Asn-Asn-Asn-Arg-Gln-Val (SEQ ID NO:1), wherein said SDF-1 fragment is less than 60 amino acids in length; fused via a covalent bond to an amino acid sequence comprising a fragment of RANTES comprising the amino acid sequence Lys-Asn-Arg-Gln-Val (SEQ ID NO:3), wherein said RANTES fragment is less than 55 amino acids in length.

110. A pharmaceutical composition comprising the chimeric protein of claim 109 in an amount effective to inhibit HIV infection or replication; and a pharmaceutically acceptable carrier.

111. A method of treating or preventing HIV infection or replication in a human subject comprising administering to the subject in which such treatment or prevention is desired the pharmaceutical composition of claim 110.

112. A chimeric protein comprising a fragment of SDF-1 comprising the amino acid sequence Cys-Ala-Leu-Gln-Ile-Val-Ala-Arg-Leu-Lys-Asn-Asn-Asn-Arg-Gln-Val-Cys (SEQ ID NO:2), wherein said SDF-1 fragment is less than 60 amino acids in length; fused via a covalent bond to an amino acid sequence comprising a fragment of RANTES comprising the amino acid sequence Cys-Ser-Asn-Pro-Ala-Val-Val-Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys (SEQ ID NO:4), wherein said RANTES fragment is less than 55 amino acids in length.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/05987

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1, 85.2, 185.1, 192.1, 195.11; 435/5, 7.2, 7.24; 514/44; 530/351, 395; 536/23.4, 23.5.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	COCCHI et al. Identification of RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$ as the major HIV-suppressive factors produced by CD8 <sup>+</sup> T cells. Science. 15 December 1995, Vol. 270, pages 1811-1815, see entire document.	1-5, 8-15, 53, 92-95 ----- 6-7, 16-52, 54-91, 96-114
X -- Y	MURPHY, P.M. The molecular biology of leukocyte chemoattractant receptors. Ann. Rev. Immunol. 1994, Vol. 12, pages 593-633, see entire document, particularly Figures 1 and 2.	38-39, 43-44, 50-52 ----- 1-37, 40-42, 45-49, 53-114

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

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# INTERNATIONAL SEARCH REPORT

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IPC (6): A61K 31/70, 38/08, 38/10, 38/19, 38/20; C07H 21/04; C07K 9/00, 14/52, 14/54; C12Q 1/70

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL : 424/85.1, 85.2, 185.1, 192.1, 195.11; 435/3, 7.2, 7.24; 514/44; 530/351, 395; 536/23.4, 23.5.

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